Why Worry About DNA?

- Cellular DNA in products might contain:
  - Cancer cell genes
  - Viral genes
- Cellular DNA in products might result in:
  - Oncogenic event
  - Pathology

Elements of DNA Risk

- Infection
- Insertional mutagenesis, activation, inactivation, up-regulation, down-regulation
- Tumor induction
  - Expression of oncogene
  - Activation of proto-oncogene(s)
  - Inactivation of tumor suppressor gene(s)

Cell Substrates
Decisions & Developments: 1954-2004

Year	Meeting	Major Outcome
1954	AF Epidemiology Board	1^{o} monkey kidney
1967	NIH	Consider human diploid cells
1978	NIH	Consider alternate cell substrates (Namalwa for Interferon)
1984	NIH/FDA	DNA, viruses, transforming proteins 10pg DNA/dose
1986	WHO Study Group	DNA, viruses, transforming proteins. 100pg DNA/dose
1996	WHO ECBS	10 ng DNA/dose
1999	FDA, NIH, WHO, IABs	DNA risk issues unresolved

1984 DNA Recommendations

"Procedures for production of biologicals must demonstrate that ***no*** cellular or other unwanted ***DNA molecules*** will be ***in the final product at a level*** which would have a biological activity. That is, activities ***which could induce changes of normal cellular processes. Until more information on the determination of the biological activities of DNA becomes available, a level of unwanted DNA in the pg range per dose appears acceptable.*** There were discussions about specific quantities of DNA that might be acceptable, and then, as an example, the currently accepted level of ***ten picograms of DNA per dose*** of polio virus produced in VERO cells ***was given as an example*** of the sort of thing that would be a good acceptable range."

Cell Substrates – 1986 WHO Study Group

- CCLs acceptable in principle
- Primary concern is viral safety
  - Emphasis on the elimination of potential viruses pathogenic for humans
- DNA of lesser concern – 100 pg
- Validation & wide margin of safety

FDA/NIAID/IABs Conference - 1999

- Cell substrate review
- No consensus on DNA issues

Impact of uncertainty & inconsistency on product improvement

- Rabies vaccine
  - Sheep brain
  - BHK-21, VERO
  - DNA

Impact of uncertainty & inconsistency on new product development

- Focus on cell characteristics
  - Lower risk cells *vs* higher risk cells
- Focus on manufacturing process
  - Address elements of risk related to cells
- Inconsistent approaches among regulatory agencies

A Way Forward

- What do we know now about the issue?
- What can we conclude from what we know now?
- What more information, if any, is needed to provide updated guidance?
- How do we get to a consensus and updated guidance?

What's Known About DNA Risk?

- Cellular DNA Can Transform Cells
  - 3T3 assays
    - 2/26 human tumor DNA scored (+)
    - Normal mouse and human DNA scored (+)
    - High MW DNA required ($30x10^6$)
    - Large amount of DNA required (20 µg)
    - Facilitator required for DNA uptake

What's Known About Cell DNA Risk?

- No evidence that cell DNA can cause Tumors
  - 250 µg hybridoma DNA negative in mice & rats
  - 100 µg HeLa DNA negative in ATS newborn rat assay
  - 10 µg T-24 DNA negative in ATS newborn rat assay
  - ~1mg T-24 DNA (i.m., i.c., i.v.) negative in immunosuppressed Rh monkeys (> 8 year followup)
  - Daily human burden of ~1 ng proto-oncogene (Temin)

What's Known About DNA Risk?

- Human Exposure to Tumor Cell DNA
  - Adeno / HeLa
  - Tumor cell transplants (1960s)
  - Blood transfusions
    - Followup of recipients of blood from donors who later developed a lymphoid cancer (75- 450 μg DNA per unit of blood)
  - Melacine: lysate of 2 melanoma cell lines
    - >1,000 patients in Phase 2 & Phase 3 studies
  - Canvaxin: 3 irradiated melanoma cell lines
    - >3,000 patients in Phase 2 & Phase 3 studies
  - Onyvax-P: 3 irradiated prostate cell lines
    - >50 in Phase 1 & Phase 2 studies

What's Known About DNA Risk?

- Human Exposure to Other DNA
  - DNA vaccines
  - Gene therapy
  - Food / GI exposure
  - Fetal DNA in maternal circulation
    - 3 to 300 fetal genomes/ml maternal plasma
    - < 313 BP in most of 23 pregnant women

What can we conclude?

- Consistent negative experimental results
- Consistent results of theoretical calculations
- If risk exists, it is vanishingly small

Past Analyses/Conclusions

Probability of an oncogenic or infectious events (100 pg DNA)

- 1986 WHO Study Group — $1 / 2 \times 10^{10}$
  - 100 pg / dose
- 1987 Regan — $1 / 10^{10}$
- 1990 Temin — $1 / 10^{12}$
- 1995 Kurth — $1 / 10^{12}$
- 1997 Dortant et al — $1 / 5 \times 10^{8}$
- 1999 Krause / Lewis — $1 / 4 \times 10^{9}$

The desire for more information

- Essential for decision making
- Nice to have

How do we move forward?

- Conference conclusions & recommendations
- ICH

Cell Substrates - Summary

- 50 years of experience
  - Primary monkey kidney → SV40
  - Human diploid → - 0 -
  - Continuous cell lines → - 0 -
  - Tumor cell DNA hasn't caused tumors *in vivo*
- Tools are available to identify risk factors
- Technology is available to address risk factors
- Rigorous cell characterization
- Extensive vaccine characterization
- Special studies specific for the cell & vaccine
- Level of risk is a function of the underlying assumptions
- **Any cell type should be acceptable for vaccine production when it has been well-characterized and shown to be free of virus or viral genes that present a risk to humans**

And the end of all our exploring

Will be to arrive where we started

And to know the place for the first time..

HeLa => 1^{o} => HDC => CHO => HeLa